REMARKS

Applicants are amending their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants are amending claim 1 to recite that the two or more target genes are derived from different samples. Note, for example, the third full paragraph on page 6 of Applicants' specification. In addition, Applicants have amended claim 15 to recite two or more types of "probes", rather than two or more types of "process", to correctly recite that the "probes" have the fluorophores, consistent with claim 1 and the discussion, for example, in the second paragraph on page 7 of the Amendment filed March 3, 2008.

Initially, it is respectfully requested that the present amendments be entered. Noting the portion of page 6 of Applicants' specification previously referred to, it is respectfully submitted that the present amendments to claim 1 do not raise any issue of new matter; and, moreover, noting, for example, the second full paragraph on page 29 of the Office Action mailed June 23, 2008, it is respectfully submitted that the present amendments to claim 1 do not raise any new issues. Moreover, by amending claim 15 to recite that the two or more types of "probes" have the fluorophores", it is respectfully submitted that the subject matter of claim 15 is clear, and that this amendment of claim 15, as well as amendments to claim 1, materially limit issues remaining in connection with the above-identified application. Noting comments made for the first time by the Examiner in the last two paragraphs on page 29 of the Office Action mailed June 23, 2008, with respect to the two or more target genes being derived from different samples and the melting temperatures; and, moreover, noting that claim 15 was a new claim in the Amendment filed March 3, 2008, it is respectfully submitted that the present amendments are clearly timely.

In view of the foregoing, it is respectfully submitted that Applicants have made the necessary showing under 37 CFR 1.116(b)(3); and that, accordingly, entry of the present amendments is clearly timely.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner patentably distinguish over the teachings of the references applied by the Examiner in the Office Action mailed June 23, 2008, in rejecting claims as formerly in the application, that is, the teachings of Ovyn, et al. (which the Examiner refers to as U.S. Patent No. 6,101,681, but which apparently should be U.S. Patent No. 6,110,681), and the articles by Whitcombe, et al., "A homogenous fluorescence assay for PCR amplicons: Its application to real-time, single-tube genotyping", in Clinical Chemistry, 44:5 (1998), pages 918-923; Rizzo, et al., "Chimeric RNA-DNA molecular beacon assay for ribonuclease H activity", in Molecular and Cellular Probes (2002) 16, 277-283; Leone, et al., "Molecular beacon probes combined with amplification by NASBA enable homogenous, real-time detection of RNA", in Nucleic Acids Research, 1998, Vol. 26, No. 9, pages 2150-2155 (Leone '98); Leone, et al., "Direct detection of potato leafroll virus in potato tubers by immunocapture and the isothermal nucleic acid amplification method NASBA", in Journal of Virological Methods 66 (1997), pages 19-27 (Leone '97); Uematsu, et al., "Real-time detection of PCR products for comparative analysis of expressed genes using module-shuffling TaqMan Probes (MTPs)", in Nucleic Acids Research Supplement No. 2, 211-212 (August 9, 2003) (as characterized by the Examiner "Uematsu 2002"), and Uematsu, et al., "Multiplex polymerase chain reaction (PCR) with color-tagged module-shuffling primers for comparing gene expression levels in various cells", in Nucleic Acids Research, 2001, Vol. 29, No. 16, pages 1-6 (August 15, 2001) (as characterized by the Examiner, "Uematsu 2001"), under the provisions of 35 USC 102 and 35 USC 103.

The Examiner's reference to U.S. Patent No. 6,101,681, in connection with Ovyn, et al., on, for example, pages 9, 10 and 17 of the Office Action mailed June 23, 2008, is noted. However, U.S. Patent No. 6,101,681 is a U.S. Patent to Patelli, et al., entitled "Fixed and Adjustable Carding Element for Textile Material Opening or Carding Machines". In contrast, note U.S. Patent No. 6,110,681 to Ovyn, et al. For clarification and accuracy, it is respectfully requested that upon further examination of the above-identified application the Examiner indicate the correct patent number of the Ovyn, et al. reference applied in rejecting claims in the above-identified application.

In addition, the repeated reference to Uematsu 2002 throughout the Office Action mailed June 23, 2008, for example, on pages 15, 16, 24 and 26, are noted. In connection with these references to Uematsu 2002, it is noted that the Examiner has not used this reference as part of the formal statement of the rejection. For this reason alone, reference to Uematsu 2002 is clearly improper, in connection with these respective prior art rejections on pages 15, 16, 24 and 26 of the Office Action mailed June 23, 2008. Note In re Hoch, 166 USPQ 406, 407n.3 (CCPA 1970). If the Examiner is going to rely on the teachings of Uematsu 2002 in various of the prior art rejections, this reference must be set forth in the statement of rejection, with requirements of 35 USC 103 being satisfied in connection therewith.

In any event, and as will be shown infra, noting comments by the Examiner in the second full paragraph on page 3 of the Office Action mailed June 23, 2008, submitted herewith is an English translation of Japanese Patent Application No. 2003-114721 (114721/2003) filed on April 18, 2003, together with a Statement as to accuracy of such translation, whereby Uematsu 2002 does not constitute prior art in connection with the presently claimed invention.

Thus, as acknowledged by the Examiner, e.g., on page 3 of the Office Action mailed June 23, 2008, Uematsu 2002 has a public availability date of August 9, 2003, after the filing date of the Japanese priority application of the above-identified application, that is, Application No. 2003-114721 filed April 18, 2003. As acknowledged by the Examiner on the "Office Action Summary" of the Office Action mailed December 29, 2005, in the above-identified application, Applicants have made a claim for foreign priority, and have submitted a certified copy of the priority document, for No. 2003-114721. Moreover, and as indicated previously, enclosed herewith please find an English translation of No. 2003-114721, together with a Statement as to accuracy thereof. Accordingly, it is respectfully submitted that all procedural requirements of 35 USC 119 and 37 CFR 1.55 have been satisfied, in connection with Applicants being accorded benefit of a filing date of No. 2003-114721.

In addition, it is respectfully submitted that substantive requirements of 35 USC 119, including requirements of the first paragraph of 35 USC 112, are satisfied by the aforementioned Japanese patent application. In this regard, note, for example, pages 6-9 of the enclosed English translation. Note also the Examples in the enclosed English translation, beginning on page 22 thereof.

Noting that the Examiner applied Uematsu 2002 for the first time in the Office Action mailed June 23, 2008, it is respectfully submitted that the presently submitted English translation together with Statement of accuracy thereof should clearly be entered, notwithstanding Finality of the Office Action mailed June 23, 2008, as submission of this English translation with Statement of accuracy clearly materially limits issues remaining in connection with the above-identified application and present submission thereof is timely.

In view of the foregoing, reconsideration and withdrawal of all rejections relying on Uematsu 2002 are respectfully requested. That is, it is respectfully submitted that the rejections set forth on pages 3-10 of the Office Action mailed June 23, 2008, relying on Uematsu 2002 as the primary reference, must be withdrawn. Moreover, it is respectfully submitted that in view of reliance on Uematsu 2002 in rejection of claims 15 and 16 on page 15; as well as reliance on Uematsu 2002 in rejection of claims 15 and 16 on page 24, of the Office Action mailed June 23, 2008, these rejections must also fall.

In any event, it is respectfully submitted that the references which are properly prior art, as applied by the Examiner, would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, having processing steps as in claim 1, including, inter alia, subjecting a gene to be analyzed to nucleic acid amplification using, inter alia, a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene being further defined, and a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore and at another end with a quencher, reverse transcriptase, RNA polymerase and ribonuclease H and/or exonuclease, with two or more target genes being simultaneously detected in a single reaction vessel using two or more types of probes, the two or more target genes being derived from different samples, and wherein each of the two or more types of probes comprise several module sequences of three or four bases, both of the terminal bases of each module sequence being identical to each other and each probe being constituted by rearranging the order of the module sequences having identical terminal bases. See claim 1.

As discussed further infra, according to the present invention each of the two or more probes used in the present invention include several module sequences of three or four bases, both of the terminal bases of each module sequence being identical to each other, and each probe being constituted by rearranging the order of the module sequences having identical terminal bases. By this specific structure of the probes, while the entire sequence of each probe is different from each other, these probes have substantially the same Tm (melting temperature) value, and can hybridize to their complementary sequences with the same reaction properties, and therefore they can be allowed to simultaneously react in a same reaction tube. Moreover, accurate quantitative analysis of two or more different target genes, even from different samples, is achieved by the present invention. Accordingly, accurate analysis can be made when the probes recited in the present claims are used for quantitative analysis.

Furthermore, it is respectfully submitted that the proper prior art references applied by the Examiner would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, having features as discussed previously in connection with claim 1, and, in addition, wherein such at least two types of probes have substantially the same melting temperature. See claim 9.

In addition, it is respectfully submitted that the teachings of the proper prior art applied references would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as discussed previously in connection with claim 1, and, moreover, wherein the two or more types of probes respectively have fluorophores at one end that emit light at fluorescent wavelengths different from each other (see claim 15); and/or wherein a

number of module sequences constituting each probe is in a range of 5-8 (see claim 16).

Moreover, it is respectfully submitted that the teachings of the proper prior art references applied by the Examiner would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as in claim 1 as discussed previously, and, additionally, having features as in the remaining dependent claims being considered on the merits, including (but not limited to) wherein a gene to be analyzed is cDNA including the first and second base sequences introduced therein by introduction with subjecting mRNA of the target gene to reverse transcription using the primer for introduction, as in claim 2; and/or wherein the nucleic acid amplification is conducted by steps as in claims 3 and 4; and/or wherein the nucleic acid amplification is conducted at a substantially single temperature (see claim 5), in particular, where such single temperature is between 37°C and 55°C (see claim 6); and/or wherein the RNA polymerase and the second base sequence are as set forth in claim 7; and/or wherein the probe is a DNA/RNA hybrid strand (see claim 14).

By use of the primer for introduction as in the present claims, which includes the first, second and third base sequences relatively located to the 5' end of the primer, with the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, the first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, together with the probe comprising a base sequence identical or complementary to the first base sequence, a universal probe for expressed gene analysis which does not have to be designed for each use in accordance with the base sequence of the target gene is achieved. The universal probe according to the present invention can

amplify and detect any type of target gene under substantially the same conditions, can detect two or more target genes derived from different samples, and analysis thereof can be simply conducted. Note, for example, the second and third paragraphs on page 6 of Applicants' specification; see also the paragraph bridging pages 28 and 29 thereof.

Furthermore, by using at least two types of probes as in the present claims, both of the terminal bases of each module sequence being identical to each other, and each probe being constituted by rearranging the order of the module sequence having additional terminal bases, the entire sequence of each probe is different from each other, but can have the same melting temperature value (note especially claim 9), and can hybridize to their complementary sequences with the same reaction properties, and therefore they can be allowed to simultaneously react in the same reaction tube, whereby accurate analysis can be made in a same reaction tube, of two or more target genes using two or more types of probes, even where the target genes are from different samples. Thus, accurate analysis can be made, when the probes are used for quantitative analysis.

Whitcombe, et al. discloses a method whereby a single TaqMan™ probe can be used for many polymerase chain reactions. The principal aim of the study reported in this article was to identify a means that the Amplification Refractory Mutation System (ARMS) could be exploited in a homogenous, high throughput and, in particular, an economical manner, and this article discloses that what was required was a way of using a single pair of allele-specific fluorescent probes for any bi-allelic polymorphism. For the fluorescent signal generation method, the authors of this article chose TaqMan™. The system used, as reported in the article, a 5'-exonuclease assay of amplicon annealed fluorogenic probes that operate in conjunction with the Amplification Refractory Mutation System, whereby relative

changes in reporter fluorescent emission are monitored in real-time using an analytical thermal cycler, this system being called Three-STAR, and it is universal in that it can either use a single probe for the detection of any one target DNA sequence or a single pair of probes for genotyping any bi-allelic polymorphism. Note the first paragraph in the left-hand column on page 918, as well as the paragraph bridging pages 918 and 919. Fig. 1 shows the scheme of the Three-STAR cycle. Note also the paragraph bridging the left- and right-hand columns on pages 921, describing that the authors have devised a way to make TaqMan™ generic inasmuch as that just one fluorogenic probe can be universally applied in any PCR reaction. See also Fig. 2 on page 921, showing single-tube genotyping.

It is respectfully submitted that Whitcombe, et al. discloses a probe non-specific to a target gene, and genotyping of 2 or more target genes in a single tube (see Fig. 2 and Table 1 on page 919). However, as recognized from the descriptions "homozygote" and "heterozygote" in the legend of Fig. 2, Whitcombe, et al. conducts typing of allele in a single sample. In contrast, two or more different target genes, derived from different samples, are detected in the present invention, using two or more probes having substantially the same Tm value. As described below, two or more probes shown in Table 1 of Whitcombe, et al. have different Tm values.

Thus, Tm values were calculated using the most accurate method, base-stacking Tm calculation (http://www.promega.com/biomath/default.htm). The FAM probe and TET probe, in Fig. 2 of Whitcombe, et al., are shown below, and respectively have melting temperatures of 65°C and 72°C:

FAM probe: CTGG CATC GGTA GGGT AAGG ATCG GTAT CG, 30mer, 65°C; and
TET probe: CGGT GGAC GTGA CGGT ACGA CGAG GCGA CG, 30mer, 72°C.

When Tm values are calculated using more simple methods, Basic Tm Calculations (66°C and 71°C) and salt-adjusted Tm calculations (61°C and 66°C)

show that these two probes of Whitcombe, et al., i.e., FAM probe and TET probe, have different Tm values. Moreover, from the FAM probe and TET probe as set forth in the foregoing, it is clear that the two probes used in Whitcombe, et al. do not have the structure of the probes as recited in the present claims. Thus, it is respectfully submitted that Whitcombe, et al. would have neither disclosed nor would have suggested various aspects of the present invention, including, inter alia, the probes used, and advantages due thereto.

Even taking into account the teachings of Ovyn, et al., and Uematsu '2001 it is respectfully submitted that the combined teachings of Whitcombe, et al., Ovyn, et al. and Uematsu '2001 as applied by the Examiner would have neither taught nor would have suggested the presently claimed subject matter, including, inter alia, the probes as recited in the present claims and as used in the presently claimed process, and target genes.

Ovyn, et al. discloses oligonucleotides that can be used as primers to amplify a region of the 16S rRNA of *Mycoplasma pneumoniae*. See column 3, lines 39-45 of this patent. Note especially column 7, lines 5-28, of this patent, describing a method for the detection of the specified microorganism. Note also column 3, lines 39-46; column 5, lines 46-67; and column 6, lines 12-16 and 43-61.

Uematsu '2001 reports on the development of a new method that can analyze plural genes from various sources by utilizing color-selective detection coupled with size separation. The color-selective detection distinguishes the gene sources and gel electrophoresis separates the gene species. The number of fragment species that can be distinguished in an electropherogram is 10-15 by use of color detection. This article discloses that the described method is the first to use module-shuffling primers (MSPs) for comparing gene expression levels in various cells. Note the last paragraph in the right-hand column on page 1 of this article. Note also the

procedure for comparing expressed genes from different sources, set forth in the second paragraph in the left-hand column on page 2 of this article. Note also the paragraph bridging the left- and right-hand columns on page 3, as well as the sole full paragraph in the right-hand column on page 3, of this article.

Even assuming, arguendo, that the teachings of Whitcombe, et al., Ovyn, et al. and Uematsu '2001 were properly combinable, such combined teachings would have neither disclosed nor would have suggested a procedure as in the present claims, including the two or more types of probes used, as recited in the present claims, with two or more target genes, derived from different specimens, being simultaneously detected in a single reaction vessel using these probes, whereby accurate quantitative analysis of the two or more target genes, derived from different specimens, can be achieved. In this regard, it is respectfully submitted that even were the teachings of the references as applied by the Examiner properly combinable, accurate quantitative analysis of two or more different target genes, derived from different specimens, cannot be achieved from the teachings thereof. Such feature would have neither been taught nor would have been suggested by the combined teachings of Whitcombe, et al., Ovyn, et al. and Uematsu '2001.

In the last paragraph on page 14 of the Office Action mailed June 23, 2008, the Examiner contends that Uematsu '2001 teaches that where each of the two or more types of probes include several module sequences of three or four bases, both of the terminal bases of each module sequence are identical to each other, and each probe is constituted by rearranging the order of the module sequences having identical terminal bases, the Examiner relying on Fig. 1 of Uematsu '2001. However, in Fig. 1 of Uematsu '2001, the primers are comprised of modules constituted of rearranged order of the module sequences. To emphasize, Fig. 1 of Uematsu '2001 shows primers, which are different from probes. Unlike probes, primers are

extended by DNA polymerases. Fig. 1 clearly states that they are "Module-shuffling Primers (MSPs)". Each of the primers shown in Fig. 1 is labeled with a fluorophore only at its 5'-terminal. It is respectfully submitted that the primers shown in Fig. 1 of Uematsu '2001 cannot work as a TaqMan probe or a molecular beacon, in which both 5'- and 3'-ends are required to be labeled with fluorophores, or one of the ends is labeled with a fluorophore and the other is labeled with a quencher. It is respectfully submitted that it is not possible to employ the primers in Fig. 1 of Uematsu '2001, as a probe.

Accordingly, it is respectfully submitted that the teachings of Whitcombe, et al., Ovyn, et al. and Uematsu '2001, as applied by the Examiner, would have neither disclosed nor would have suggested features of the present invention as discussed previously, including the probes employed.

In connection with claim 14, the article by Rizzo, et al. discloses preparation of RNA/DNA chimeric molecular beacons, which contain a single-stranded RNA/DNA chimeric oligonucleotide labeled with a 5'-fluorescein as fluorophore and a 3'-DABCYL as quencher, referring to Fig. 1 on page 279 of this article. This article discloses that the fluorophore of the probe is held in proximity to the quencher by the stem-loop structure; and that when the RNA sequence of the RNA/DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested. Note the second full paragraph in the left-hand column on page 278 of this article. Note also the paragraph on pages 279 and 280; and the Conclusion set forth in the left-hand column on page 282, of this article.

Even assuming, arguendo, that the teachings of Rizzo, et al. were properly combinable with the teachings of Whitcombe, et al., Uematsu '2001, and Ovyn, et al., as applied by the Examiner, such combined teachings would have neither disclosed nor would have suggested the presently claimed method, including the two

or more types of probes used, with two or more target genes, derived from different samples, being simultaneously detected in a single reaction vessel, and advantages thereof, as discussed previously, and/or such feature together with at least one of the probes being a DNA/RNA hybrid strand, as in claim 14.

It is respectfully submitted that the combined teachings of Whitcombe, et al., Uematsu '2001, and Leone 1998 (as evidenced by Leone 1997) would have neither disclosed nor would have suggested the presently claimed method.

Whitcombe, et al. and Uematsu '2001 have been previously discussed.

Leone 1998 discloses employment of molecular beacon probes in a NASBA amplicon detection system to generate a specific fluorescent signal concomitantly with amplification. This article describes the coupling of RNA amplification by NASBA with amplicon detection by molecular beacon technology to produce a homogenous assay, called AmpliDet RNA. Note the first full paragraph in the left-hand column on page 2151 of this article. See also the discussions under the headings "Selection of amplification primers and probe", "Synthesis of the molecular beacons", "NASBA" and "Post-NASBA analysis", on page 2151 of this article.

Leone 1997 contains a description that the sense primers were entirely target specific, wherein the antisense primers consisted of a 3' terminal, target specific sequence and a 5' terminal T7 promoter sequence. See page 21, Section 2.2.

Even assuming, arguendo, that the teachings of Whitcombe, et al. and Uematsu '2001 were properly combinable with the teachings of the two Leone articles, such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including wherein two or more target genes, derived from different samples, are simultaneously detected in a single reaction vessel using two or more types of probes, the probes being defined as in the present claims, including, inter alia, wherein both of the terminal bases of each

module sequence of the respective probes are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases.

Furthermore, it is respectfully submitted that the combined teachings of Whitcombe, et al., Uematsu '2001, the two Leone, et al. articles and Rizzo, et al., would have neither taught nor would have suggested the subject matter of claim 14.

The teachings of Whitcombe, et al. has been previously discussed, as has the teachings of each of Uematsu 2001, Leone 1998, Leone 1997 and Rizzo, et al. Even assuming, arguendo, that the teachings of these references were properly combinable, such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, detection of two or more target genes, derived from different samples, simultaneously in a single reaction vessel using two or more types of probes, with the two or more types of probes being further defined as in the present claims, including, inter alia, wherein both of the terminal bases of each module sequence are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases, with advantages thereof as discussed previously, including, inter alia, wherein the melting temperatures of the probes are substantially the same (see claim 9).

It is emphasized that according to features of the present invention, two or more different target genes derived from different samples are detected, using two or more probes having substantially the same Tm value. It is respectfully submitted that this is one of the characteristic features of the present invention. In contrast, it is respectfully submitted that Whitcombe, et al. merely described typing of an allele in a single sample. Even in light of the teachings of Leone 1998 and Leone 1997, the teachings of the applied references would have neither disclosed nor would have

suggested the use of two or more probes having substantially the same Tm value, or wherein two or more different target genes derived from different samples are detected, and advantages thereof.

In the second full paragraph on page 29 of the Office Action mailed June 23, 2008, the Examiner contends that features upon which Applicants rely, that is, two or more target genes being derived from different samples, "are not recited in the rejected claim(s)". Such contention is moot, in light of present amendments to the claims to recite, in the sole independent claim being considered on the merits, that the two or more target genes are derived from different samples.

The contention by the Examiner in the last two lines on page 29 of the Office Action mailed June 23, 2008, that the recitations directed to the module shuffling "have been addressed by the inclusion of Uematsu 2001 and 2002", is noted. In view of overcoming Uematsu '2002 as prior art, by perfecting Applicants' claim of foreign priority, it is respectfully submitted that the Examiner has not established that such features of the present invention would have been known or suggested prior to the present invention.

The additional contention by the Examiner in the last paragraph on page 29 of the Office Action mailed June 23, 2008, that the melting temperatures of 65°C and 72°C in the prior art is interpreted to be "substantially the same", is respectfully traversed. It is respectfully submitted that the temperature gap of 7°C has an effect on amplification conditions. When the melting temperature changes from 65°C to 72°C, different amplification conditions due to this temperature change must be considered. Therefore, the difference in temperature of 65°C to 72°C is not "substantially the same", contrary to the contention by the Examiner. It is respectfully submitted that in the field of gene amplification with the use of polymerase chain reaction, suitable amplification conditions must be considered

when a melting temperature is shifted by a margin of 1 or 2°C. Clearly, such change of 7°C is not "substantially the same" temperature as in various of the present claims.

In view of the foregoing comments and amendments, and in view of the presently submitted English translation (together with Statement of accuracy) of Japanese Patent Application No. 2003-114721, entry of the present amendments and of the aforementioned English translation (with Statement of accuracy), and reconsideration and allowance of all claims presently being considered on the merits in the above-identified application, are respectfully requested.

Kindly charge any shortage in fees due in connection with the filing of this paper to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Deposit Account No. 01-2135 (case 1021.43414X00), and credit any excess payment of fees to such Deposit Account.

Respectfully submitted,

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Attachment: English translation of Japanese Patent Application No. 2003-114721

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